

UNITED STATES PATENT APPLICATION

For

SHAPED ILLUMINATION GEOMETRY AND INTENSITY USING A DIFFRACTIVE
OPTICAL ELEMENT

by

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I. FIELD OF THE INVENTION

[001] This invention relates to methods and optical systems for illuminating a target. The present invention also relates to methods and systems for performing sample assays, and for producing and measuring optical responses and signatures.

II. BACKGROUND OF THE INVENTION

[002] Targets, such as areas where optically transduced chemical and/or biochemical assays are performed, may need to be illuminated by a light source. It is often desirable to illuminate the target with light having enhanced uniformity of intensity over the entire target region. Optical signals are typically a function of the illumination intensity and, the more an illumination intensity varies across a target, the more the optical signal will also vary. The resultant variance in optical signals may be undesirable.

[003] However, it can be difficult to efficiently provide illumination having enhanced or a high degree of uniformity. For example, lasers, which are commonly used for illuminating targets, typically have an intensity profile that is peaked at its center and which drops off radially towards the edges. This intensity profile is often a Gaussian, or bell shaped, profile. Therefore, if a target is directly illuminated with such a laser, the illumination of the target will not have a constant intensity. Rather, the center portion of the target will receive greater illumination intensity than the perimeter areas.

[004] Therefore, there is a need for an apparatus and method to illuminate a target or selected area with enhanced uniformity as compared to directly illuminating the target with a given light source. Further, there is a need for an apparatus and

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method that provides enhanced illumination uniformity for optical targets such as those in chemical and/or biochemical assay systems.

III. SUMMARY OF THE INVENTION

[005] According to certain embodiments of the invention, an apparatus is provided to illuminate a target. The apparatus comprises a light source, a first lens, a diffractive optical element, and a second lens. The first lens is configured to receive light from the light source. The diffractive optical element is configured to receive the light from the first lens and to regulate the light into regulated light. The second lens is configured to receive the regulated light and to direct the regulated light onto a selected area of the target.

[006] According to certain embodiments of the invention, a method is provided to illuminate a target. The method comprises generating light from a light source, directing the light with a first lens to a diffractive optical element, generating regulated light with the diffractive optical element, and focusing the regulated light with a second lens onto a selected area of the target.

[007] According to yet another aspect of the present invention, the inventive apparatus and method provide non-normal angle of incidence illumination of a selected area with a given light source with a greater degree of uniformity than is achieved when that light source is used to directly illuminate the selected area at the same non-normal angle of incidence.

[008] According to certain embodiments of the present invention, the inventive apparatus and method are directed towards the analysis of a sample in which light is generated from a light source, the light is directed with a first lens to a diffractive

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optical element; regulated light is generated with the diffractive optical element; the regulated light is delivered onto a selected area of a target that comprises at least one optically active species; and changes in an optical signature of the at least one optically active species are detected.

[009] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

[010] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the invention.

[011] Figure 1 is a schematic illustration of one embodiment of an apparatus of the present invention.

[012] Figure 2 is a schematic illustration of the illumination of a selected area at a non-normal angle of incidence, where the regulated light has a gradient intensity profile in order to provide substantially uniform illumination of the selected area at a non-normal angle of incidence.

[013] Figure 3 A and B illustrate the distortive effect of non-normal angle illumination of a selected area and the use of regulated light to compensate for this effect in order to more uniformly illuminate a selected area of a given shape.

[014] Figure 4 is a schematic illustration of an embodiment according to the present invention, having an optical diffuser for removing speckle arranged between the first lens and the diffractive optical element.

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[018] The relative deviation of the intensity can be found from the ratio of the standard deviation of the intensity to the mean value of the intensity, each measured within the selected region. The relative deviation may also characterize a scaled intensity variation, which is calculated from the ratio of the standard deviation of a scaled intensity to the mean value of the scaled intensity, each measured within the

is greater than the illumination uniformity that results from the direct illumination of the selected region with the given light source. For example, if the light source is a laser that emits light with a Gaussian intensity distribution, the uniformity of the substantially uniform illumination at the selected region is greater than a Gaussian distribution. Likewise, if the light source has a radial intensity distribution of $1/r$, the uniformity of the substantially uniform illumination at the selected region is greater than a $1/r$ distribution.

[022] The light intensity uniformity for "substantially uniform illumination" and "substantially uniformly illuminate" may also be scaled by the shape, including the depth, of the selected region. Thus, for example, if the selected region includes one or more wells with a curved depth profile, the uniformity of the substantially uniform illumination can be matched to the curved profile and, for example, provide more intense illumination where the well is deeper and less intense illumination where the well is shallower. In this case, if the intensity distribution is scaled by, for example, the variable depth of the selected region, the scaled intensity distribution will be substantially uniform, meaning that it is at least greater than the uniformity of the light intensity as initially emitted from the light source when similarly scaled.

[023] The terms "intensity profile" and "light intensity distribution" as used herein in reference to light, refer to the distribution of optical intensity in the cross-sectional area of the light, where the cross-section is perpendicular to the light's propagation axis. As is the case with illumination, the intensity profile may have a given degree of uniformity, as measured, for example, by the relative deviation of the optical intensity (i.e., intensity variation) in the cross-sectional area. An intensity profile may

number," as used herein, is the expression denoting the ratio of the equivalent focal length of a lens to the diameter of its entrance pupil, and is equivalent to $1/(2 \text{ NA})$, where NA is the numerical aperture. "Depth of field," as used herein, refers to the distance range over which light can be focused on a given subject while providing adequate definition and/or clarity.

[026] The term "edge sharpness," as used herein, refers to the change in intensity at the edge of the region selected for illumination. According to certain embodiments of the present invention, the edge sharpness optionally may be selected to be greater than the edge sharpness of the light as emitted from the light source. For example, if the light source is a laser source emitting light with a Gaussian intensity distribution, the edge sharpness of the regulated light optionally may be selected to be greater (that is, change more quickly at the edge of the selected region) than the originally emitted light. Additionally, the regulated light may have, for example, an edge sharpness that is approximately square shaped. The regulated light may have, for example, an edge sharpness that is matched to the shape, including the depth, of the selected region. Thus, for example, if the selected region includes one or more wells with curved depth profiles, the edge sharpness optionally can be matched to the curved profile of the one or more wells in order to provide more intense illumination where the well is deeper and less intense illumination where the well is shallower. Of course, the inverse intensity distribution (that is, more intense where the well is shallow and less intense where it is deep) is also possible.

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configured to receive light from the light source. The diffractive optical element is configured to receive the light from the first lens and to regulate the light into regulated light. The second lens is configured to receive the regulated light and to direct the regulated light onto a selected area of the target.

[034] According to certain embodiments, the present invention provides a method to illuminate a target. This method comprises generating light from a light source, directing the light with a first lens to a diffractive optical element, generating regulated light with the diffractive optical element, and focusing the regulated light with a second lens onto a selected area of the target.

[035] Figure 1 schematically illustrates an apparatus according to certain embodiments of the invention. Light source 10 emits light 20, which is received by the first lens 30. The light 20 is directed by first lens 30 to the diffractive optical element 40. In these illustrative embodiments, the light 20 emitted from the light source 10 is divergent with an angle of divergence α , and has an intensity profile that is brightest in the center, as shown by the shading profile, which is darkest at the center. In these illustrative embodiments, the first lens 30 is also configured to collimate the light 20, as shown schematically.

[036] The diffractive optical element 40 receives the light 20 and regulates it into regulated light 50. In this illustrative embodiment, the regulated light 50 has an intensity profile that is substantially uniform, as shown by the substantially uniform shading profile. In these embodiments, the diffractive optical element 40 is also configured to reshape the light 20 from an elliptical shape into rectangular shaped regulated light 50. The second lens 60 receives the regulated light 50 and directs it

onto a selected area 75 of the target 70. In this illustrative embodiment, the second lens 60 is configured to focus the regulated light 50 towards the selected area 75, as shown schematically.

[037] In certain embodiments, the type and properties of the light source, first lens, diffractive optical element, and second lens may be chosen together, such that the desired shape, size, and/or uniformity is achieved for illumination of the target.

Additional considerations for each of these elements will also be discussed below.

[038] The light source may be any effective light source that, functionally, emits light. In certain embodiments, characteristics of the light source may include one or more characteristic chosen from, for example, a fixed or tunable output wavelength or wavelength range; a fixed or tunable output intensity; a stable, oscillating or pulsed output; a collimated or un-collimated output; a fixed or variable divergence; and a coherent or incoherent output.

[039] According to certain embodiments, the light source may be wholly or partially coherent or incoherent, and may be chosen from, for example, at least one of the following non-limiting examples: a laser, an electroluminescent light source, a chemoluminescent light source, an electrochemoluminescent light source, an incandescent light source, a fluorescent light source, an arc lamp, and a light emitting diode. According to certain embodiments, the light source may also be chosen from continuous wave (CW) and pulsed lasers and from gas, solid state, fiber optical, and organic based lasers.

[040] According to certain embodiments, the light source may comprise one or more light sources. For example, the light source may comprise a first light source

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substantially matched to a size of the selected area. According to certain embodiments, the second lens may also comprise an objective lens, which may or may not be further configured to collect light from the selected area. According to certain embodiments, an objective lens may be used, for example, for either one or both of the non-normal axis illumination of the selected area and the collection of light (e.g., luminescent emission) from the selected area.

[045] The first and second lenses of the apparatus may be independently chosen from any suitable optical element or combination of elements. Functionally, a lens bends light rays causing them to, for example, at least one of converge and diverge. A lens may be an object or group of objects, and may be at least one of reflective, refractive, and diffractive. According to certain embodiments, the first and second lenses may be independently selected from the following non-limiting examples: refractive optical elements, reflective optical elements, and diffractive optical elements. According to certain embodiments, the first and/or second lens may be combined integrally, such as on a surface, or non-integrally with one or more other optical elements, such as a diffractive optical element; a grating, such as a transmission grating; an optical filter; and a refractive element, such as a prism. According to certain embodiments, the first and/or second lens may be combined integrally, such as on a surface, or non-integrally with one or more other non-optical elements, such as a sample holder, a fluidic system, a mounting system, and/or a target. According to certain embodiments, the first and/or the second lens may be disposable. For example, the second lens may be integrally formed in a disposable

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configured to receive light of a given shape and intensity distribution, and to redistribute the light to produce a desired shape and/or intensity distribution. The redistribution results from diffraction of the light by the diffraction optical element. The meaning of regulated light, as used herein, is light that has been redistributed from a first cross sectional shape and/or intensity distribution to a second cross sectional shape and/or intensity distribution.

[053] According to certain embodiments, the redistribution may include wavelength dependent redistribution. In certain embodiments, for example, the relative wavelength distribution of the regulated light at a first point in its cross section may differ from at least one of (1) the relative wavelength distribution at a second point in its cross section and (2) an average wavelength distribution of the light received by the diffractive optical element.

[054] According to certain embodiments, the redistribution may include no (or minimal) wavelength dependent redistribution. In certain embodiments, for example, the relative wavelength distribution of the regulated light at a first point in its cross section does not differ (or differ significantly for the purpose of illuminating the target) from at least one of (1) the relative wavelength distribution at a second point in its cross section and (2) an average wavelength distribution of the light received by the diffractive optical element.

[055] According to certain embodiments, diffractive optical elements can give a desired intensity distribution in the diffraction plane. According to certain embodiments, these elements can be designed using a number of methods, including geometrical optics methods and iterative-Fourier-transform algorithms

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(IFTAs). IFTAs can be used to design diffractive optical elements producing any desired intensity distribution in the diffraction plane based on any intensity cross-section of the incident beam. See, for example, M. Johansson et al., "Robust design method for highly efficient beam-shaping diffractive optical elements using an iterative-Fourier-transform algorithm with soft operations," *Journal of Modern Optics*, 47(8), 1385 - 1398 (2000), the entirety of which is incorporated herein by reference for any purpose. According to certain embodiments, optical elements, and their combinations, including, for example, diffractive optical elements, can be designed using software packages, such as, for example, ZEMAX® from Focus Software, Inc.

[056] Diffractive optical elements may include, e.g., holograms and holographic optical elements. According to certain embodiments, suitable diffractive optical elements include, for example, those chosen from amplitude (e.g., absorption) and phase holograms; optically etched diffractive optical elements; embossed diffractive optical elements; molded diffractive optical elements; chemically etched diffractive optical elements; thin or surface (2-dimensional) holographic optical elements and volume (3-dimensional) holographic optical elements; reflection and transmission holograms; multiplex holograms; rotating holograms, such as, for example, a rotating disc composed of a series of holographic optical elements that diffracts light at various angles, when spinning, for example, to generate a raster scan; Fresnel holograms; and combinations thereof.

[057] According to certain embodiments, the diffractive optical element is configured to regulate the received light and compensate for at least one of light intensity distributions and shapes of the light due to at least one of the light source

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and interaction of the light with optical elements of the apparatus. As a non-limiting example, the diffractive optical element can be configured to redistribute the Gaussian intensity profile of light from a laser to another intensity profile, such as a more uniform intensity profile. As another non-limiting example, the diffractive optical element can be configured to redistribute the circular profile of light from a light source to another shape, such as a rectangular profile. As yet another non-limiting example, the diffractive optical element can be configured to compensate for aberrations, such as spherical and chromatic aberrations, such as those caused by the interaction of the light with optical elements of the apparatus.

[058] According to certain embodiments, the diffractive optical element regulates the received light, having a given degree of uniformity, such that the output light has a greater degree of uniformity at the selected area of the target. According to certain embodiments, the enhanced uniformity may be measured with respect to the intensity distribution. That is, the output light may have a more uniform intensity distribution (measured at the selected area) than the received light (if the received light were transmitted to and measured at the selected area without being regulated by the diffractive optical element).

[059] According to certain embodiments, the diffractive optical element may regulate the received light by reshaping the cross sectional profile of the light. For example, the diffractive optical element may reshape the received light from a generally circular or elliptical cross section to form regulated light having a generally rectangular cross section. Of course, the diffractive optical element may be

configured to regulate received light having shapes other than circular, and to generate regulated light having shapes other than rectangular.

[060] For example, as shown in Figure 1, light 20 having an elliptical cross-section and an intensity distribution peaked in the center (as shown by darkened central shading) is regulated by the diffractive optical element 40 and regulated into regulated light 50 that has a rectangular cross-section and a constant intensity distribution (as shown by uniform shading). Of course, as discussed above, according to certain embodiments the light need not be regulated into regulated light having a constant intensity distribution.

[061] The resultant intensity re-distributed and/or re-shaped light may or may not be immediately suitable for the illumination of the selected area of the target. For example, in certain embodiments, the diffractive optical element may be configured to not only post-compensate for effects of elements optically prior to the diffractive optical element but also to pre-compensate for effects of elements optically subsequent to the diffractive optical element. Thus, according to certain embodiments, the regulated light is most suitable for illumination of the selected area after it interacts with the second lens and/or other subsequent elements.

Additionally, according to certain embodiments, the regulated light may be designed to allow for its propagation, including any changes in size, shape, and intensity distributions that result therefrom, prior to its illumination of the selected area.

[062] According to certain embodiments, the diffractive optical element may be configured to produce regulated light that is less uniform than the received light but which, after interacting with subsequent optical elements and/or further propagation,

is more uniform when it illuminates the sample. According to certain embodiments, the diffractive optical element may be configured to produce regulated light having a cross section that is not matched (in, for example, size and/or shape) to the selected area of the target but which, after interacting with subsequent optical elements and/or further propagation, is matched (in, for example, size and/or shape) to the selected area.

[063] Certain embodiments of the apparatus may be configured to substantially uniformly illuminate the selected area without the regulated light interacting with a second lens. According to certain embodiments, such apparatuses may be advantageous to illuminate selected areas at distances that are large compared with the beam diameter. According to certain embodiments, such apparatuses may be advantageous to illuminate selected areas at distances that are at least 1.5 times as large as the beam diameter. According to certain embodiments, such apparatuses may be advantageous to illuminate selected areas at distances that are at least 2 times as large as the beam diameter. According to certain embodiments, such apparatuses may be advantageous to illuminate selected areas at distances that are at least 10 times as large as the beam diameter. According to certain embodiments, such apparatuses may be advantageous to illuminate targets comprising multiple, spatially separate selected areas.

[064] Suitable diffractive optical elements include, but are not limited to, reconfigurable holographic optics, such as those disclosed in U.S. Patent No. 6,175,431 to Waldern et al., which is incorporated by reference herein in its entirety for any purpose. Diffractive optical elements according to certain embodiments of

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[068] According to certain embodiments, the apparatus may be configured to illuminate a selected area of a given shape at a normal or non-normal angle of incidence. For example, in certain embodiments, there can be non-normal illumination. In certain embodiments, if the selected area is, for example, square shaped, the regulated light would be shaped such that, when incident on the selected area at the non-normal angle of incidence, the light illuminates a square shaped area. Due to the non-normal angle of incidence, however, the regulated light will not necessarily have a square shaped profile.

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[071] According to various embodiments, illumination can be provided with a range of intensity variations. For example, embodiments may be configured to illuminate the selected area with an intensity variation of 50% or less, including an intensity variation of 10% or less, including an intensity variation of 5% or less, including an

intensity variation of 1% or less, and including an intensity variation at any value between 50% to less than 1%. According to certain embodiments, the selection of an appropriate intensity variation may take into account any one or more of the following non-limiting factors: the intensity variation of the light source, the requirements for the target illumination, and the type of diffractive optical element employed, as well as other factors such as size, cost, and tolerance limitations of the apparatus and/or method. According to certain embodiments, the intensity variation may be a scaled intensity variation.

[072] According to certain embodiments, a range of illumination efficiencies for illumination of the selected area may be provided. For example, embodiments may be configured to direct at least 1% percent of the light from the light source onto the selected area, including at least 10% percent of the light, including at least 25% percent of the light, including at least 50% percent of the light, including at least 75% percent of the light, including at least 90% percent of the light, including at least 99% of the light and including any percent between 1% and 100%. According to certain embodiments, the selection of an appropriate illumination efficiency may take into account any one or more of the following non-limiting factors: the number and type of optical elements in the apparatus, including the light source and its intensity, and the intensity requirements for the sample illumination. In certain embodiments, one may factor in the type of power source for the system, where, for example, relatively high efficiencies may be desirable for battery powered operation.

[073] In certain embodiments, the present invention may be configured to direct at least 10% percent of the light from the light source onto the selected area and to

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illuminate the selected area with an intensity variation of less than 25%. In certain embodiments, the present invention may be configured to direct any percent between 10% and 100% percent of the light from the light source onto the selected area and to illuminate the selected area with an intensity variation of any percent less than 25%.

[074] In certain embodiments of the present invention, the apparatus may be configured to control a numerical aperture of the regulated light directed onto the selected area. In certain embodiments, such a design can be used to produce a selected depth of field and a selected edge sharpness.

[075] According to various embodiments, virtually any target or selected area may be illuminated. For example, according to certain embodiments, the target may comprise at least one optical fiber bundle, including an optical fiber bundle comprising separate wells at terminal ends of optical fibers of the bundle, and including optical fiber bundles such as those disclosed in U.S. Patent No. 6,023,540 to Walt et al., which is incorporated by reference herein in its entirety for any purpose. According to certain embodiments, the target may comprise at least one microcard, including, for example, those sold under the trade name TAQMAN® CYTOKINE GENE EXPRESSION CARDS by Applied Biosystems. According to certain embodiments, the target may comprise at least one of a substrate, fluidics network, and device, such as those disclosed in U.S. Patent No. 6,126,899; WO Application No. 97/36681; and U.S. Patent No. 6,272,939, the disclosures of which are incorporated herein by reference in their entirety for any purpose. According to

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stranded molecules. Exemplary hybridization is described in Sambrook et al., eds., *Molecular Cloning: A Laboratory Manual*, 2nd Edition, Chapter 5, Cold Spring Harbor Laboratory Press (1989); Grossman and Colburn, *Capillary Electrophoresis Theory and Practice*, Chapter 1, Academic Press (1992); *Annu. Rev. Biomed. Eng.* 3:195–223 (2001); *Proc. IEEE*, 88(12) 1949-1971 (2000); and *Annu. Rev. Genomics Hum. Genet.* 1:329–60 (2000); Alphey, *DNA Sequencing*, Chapter 7, BIOS Scientific Publishers Limited (1997), the disclosures of which are incorporated herein by reference in their entirety for any purpose. Electrophoresis is a process in which electrically charged particles that are suspended in a solution move through the solution under the influence of an applied electric field. Exemplary electrophoresis is described in Grossman and Colburn, *Capillary Electrophoresis Theory and Practice*, Chapter 1, Academic Press (1992); *Annu. Rev. Biomed. Eng.* 3:195–223 (2001); *Chem. Rev.* 99, 3081-3131 (1999); *Proc. IEEE*, 88(12) 1949-1971 (2000); *J. Biochem. Biophys. Methods* 41, 103–119 (1999); Novotny, *Capillary Electrophoresis*, *Biotechnology*, 7:29-34 (1996); and U.S. Patent Nos. 5,741,411, 6,236,945, 5,790,727, and 4,833,827, the disclosures of which are incorporated herein by reference in their entirety for any purpose.

[087] According to certain embodiments, an apparatus is provided that can illuminate a target having a target array. In certain embodiments, the apparatus comprises a target comprising a target array, a light source, a first lens configured to receive light from the light source, a diffractive optical element configured to receive the light from the first lens and to regulate the light into regulated light, and a second lens configured to receive the regulated light and to direct the regulated light onto a

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selected area of the target. A target array is understood to be a target composed of more than one sub targets. Non-limiting examples of target arrays include the multiple fiber wells disclosed in *Walt et al.*; U.S. Patent No. US 6,023,540; J.A. Ferguson *et al.*, *Analytical Chemistry*, 72, 5618 (2000); F.J. Steemers *et al.*, *Nature Biotechnology*, 18, 91-94 (2000); and D.R. Walt, *Science*, 287, 451-452 (2000), the disclosures of which are incorporated herein by reference in their entirety for any purpose.

[088] According to certain embodiments an apparatus is provided that is configured to perform an assay on a sample. In certain embodiments, the apparatus comprises a target configured to receive the sample, a light source, a first lens configured to receive light from the light source, a diffractive optical element configured to receive the light from the first lens, and to regulate the light into regulated light, and a second lens configured to receive the regulated light and to direct the regulated light onto a selected area of the target. According to various embodiments, the sample may be in any form, such as solid, liquid, gas, and mixtures thereof. Non-limiting examples of such samples include blood and samples derived from blood, samples of proteins, samples of nucleic acids, air samples, and/or solutions comprising antibodies and/or antigens. According to certain embodiments, at least one of the target and the sample comprises at least one optically active species. According to certain embodiments, at least one of the target and the sample comprises at least one fluorescent species.

[089] According to certain embodiments, the sample may comprises a "biological sample," which is used in a broad sense and is intended to include a variety of

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biological sources that contain nucleic acids. Such sources include, without limitation, whole tissues, including biopsy materials and aspirates; *in vitro* cultured cells, including primary and secondary cells, transformed cell lines, and tissue and cellular explants; whole blood, red blood cells, white blood cells, and lymph; and body fluids such as urine, sputum, semen, secretions, eye washes and aspirates, lung washes and aspirates. According to certain embodiments, samples comprising fungal and/or plant tissues, such as leaves, roots, stems, and caps, are also within the scope of the present invention. According to certain embodiments, samples comprising microorganisms and/or viruses that may be present on or in a biological sample are within the scope of the invention.

[090] According to certain embodiments, an apparatus is provided that is configured to perform an assay on a sample. According to certain embodiments, as shown in Figure 4, the apparatus comprises a target 70 configured to receive the sample. According to certain embodiments, the target may comprise a sample holder and/or a sample. The apparatus further comprises a light source 10, a first lens 30 configured to receive light 20 from the light source 10, and a diffractive optical element 40. The diffractive optical element 40 is configured to receive the light 20 from the first lens 10, to regulate the light 20 into regulated light 50, and to direct the regulated light onto the selected area 75 of the target 70. As illustrated by the gradated shading of regulated light 50, the regulated light may have an intensity gradient to provide more uniform illumination intensity (as compared with light having no intensity gradient) to the selected area 75 when illuminated at a non-normal angle of incidence, β . In certain embodiments, the regulated light 50 may be shaped with

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a shape that matches the selected area after illumination of the selected area at a non-normal angle of incidence. According to various embodiments, a second lens, configured to direct the regulated light onto the selected area, is optional.

[091] According to certain embodiments, as shown in Figure 4, the selected area 75 may be a well, such a well configured to receive a sample. According to certain embodiments, at least one of the target and the sample comprises at least one optically active species 90. According to certain embodiments, the optically active species 90 comprises at least one fluorescent species.

[092] In certain embodiments, an apparatus optionally may include an optical diffuser configured to remove speckle, such as speckle due to the interference of coherent light. According to certain embodiments, as shown in Figure 4, an optical diffuser 80 may be located between the first lens 30 and the diffractive optical element 40. The optical diffuser, however, may be placed anywhere within the apparatus. According to certain embodiments, the optical diffuser may be any suitable optical element which is useful for removing speckle. In certain embodiments, the optical diffuser may comprise at least one of a rotating optical diffuser and a light shaping optical diffuser (LSD), such as, for example, an LSD comprising surface relief holograms with random, non-periodic structures. In certain embodiments, one may use optical diffusers from Physical Optical Corporation, including those with the catalog numbers LSD-KIT-CN-x-y, where x is a diffuser angle chosen from 0.5, 1, 5, and 10° and where y is a diameter chosen from 25 and 50 mm.

[096] It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

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